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Title: Field evaluation of three topically applied insect repellent products containing IR3535 against mosquitoes in Florida.

Protocol Version 4
22nd March 2017

Study Center:
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Sponsor and Funder:
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Trial Monitoring Center:
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This protocol provides information about procedures for entering participants into repellent trials. The protocol should not be used as a guide for the treatment of others; every care was taken in its drafting, but corrections or amendments may be necessary.
Problems relating to this trial should be referred, in the first instance, to the Principal Investigator.

This trial will adhere to the principles outlined in the International Conference on Harmonization Good Clinical Practice (ICH GCP) guidelines, protocol and all applicable local regulations.

Study Synopsis

Name of Sponsor:	LivFul Inc.			
Name(s) of Product:	Three LivFul Inc. mosquito repellent products (Lotion, Spray, Wipe)			
Title of Study:	Field evaluation of three topically applied insect repellent products containing IR3535 against mosquitoes in Florida			
Short Title:	Field repellent testing in Florida			
Protocol Number:	2	**Version:**	V4.0	
Type of Product:	Spray, lotion and wipe		**Phase of Development:**	IV
Principal Investigator:	Dr. Emma Weeks, University of Florida, Gainesville, FL, USA			
Monitoring Investigator:	Arthropod Control Product Test Centre (*arctec),* London School of Hygiene & Tropical Medicine, London, UK			
Primary/Secondary Objective(s):	***Primary objective:*** To determine the efficacy and duration of three topically applied insect repellent products at preventing landing by mosquitoes. Efficacy will be determined by calculating the complete protection time (CPT), which is defined as the time between application of the repellent product and the occurrence of the first landing in a 5 minute test, followed by a confirmatory landing within 30 minutes			
Study Design / Methodology:	A two-site field setting study using healthy volunteers to test three insect repellent product formulations (lotion, spray, and wipe) against mosquitoes.			
Number of Participants:	The sample size calculations (based on 90% power and a 5% significance level) require 10 participants. Two untreated participants will also monitor the landing rate throughout the study. An additional 6 participants could be enrolled as alternatives.			
Intended Product Users:	The test product is designed for use by the general public in areas where mosquito biting is likely.			
Study Duration:	Participants will undergo a screening evaluation which includes a training (to detect mosquito landings and use an aspirator), a mosquito attraction test, and a dose determination assay. Followed by participation in up to 6 repellency tests for up to 12 hours duration. In total each of the three products is to be tested for 12 hours at each of the two field sites. Each participant will be followed up after each visit by email/in person/by phone within 72 hours of the visit to monitor for adverse events. The minimum duration of participant involvement			

	would therefore be approximately 1 week. Total study duration (recruitment and participant involvement) is anticipated to be ~ 6 months.

Inclusion Criteria:	• Able and willing to give fully informed consent; • Able to understand and comply with the study procedures; • Consider themselves to be in good general health; • Male or female; • Aged 18 to 55 years; • Non-smokers or willing to refrain for 24 hours prior to and during each test; • Willing to undergo a mosquito attraction test putting an arm into a cage of mosquitoes. • Willing to complete mosquito handling training and complete dose determination assays. • Able to understand English
Exclusion Criteria:	• Suspected or known to be sensitive or allergic to, or phobic of, mosquito bites; • Participated in an interventional study (other than an insect repellent study) in the previous 3 months; • Participated in a biting insect study in the previous 48 hours; • Aware of having any cardiovascular or respiratory disorder (whether active or inactive); • Individuals with localized skin disorders or problems affecting the legs (such as eczema, psoriasis, or atopic dermatitis) or open cuts or scrapes; • Allergic to any of the test or reference product ingredients; • Women who are pregnant, nursing or intending to become pregnant; • Previous anaphylaxis; • Aware of having a compromised immune system; • Employees, managers, and spouses of employees of the University of Florida and of the study Sponsor. • Unable to understand English

Efficacy Endpoint(s):	***Primary:*** Median Complete Protection Time for the repellent product, tested against mosquitoes for prevention of landing.
Safety Endpoint(s):	Adverse event data will be collected and summarized.
Statistical Methods:	Median Complete Protection Time will be calculated using the Kaplan Meier survival-function.
Investigation Site(s):	***Single-center:*** Entomology and Nematology Department, University of Florida, PO Box 110620 Building 970, Natural Area Dr. Gainesville, FL 32611.
Monitor:	Arthropod Control Product Test Centre (*arctec*), Chariot Innovations Limited, a wholly-owned subsidiary of the London School of Hygiene & Tropical Medicine (LSHTM).

TABLE OF CONTENTS

1. Introduction

Mosquitoes, midges, sandflies and other biting insects are vectors of extremely important diseases such as malaria, yellow fever, filariasis and many viruses and also may be of great nuisance value. The use of repellent products can provide added personal protection from disease transmission and nuisance bites. New effective repellents would offer an additional option for protection against biting insects. The tests carried out provide important information on the effectiveness of skin repellents, which will be used for label claims to accurately inform consumers and registration purposes.

The aim of this study is to provide longevity and efficacy data for three topically applied insect repellent products for prevention of mosquito landing. The product will be provided by LivFul Inc.

2. Objectives

Primary objective: To determine the efficacy and duration of three topically applied insect repellent products at preventing landing by mosquitoes over 12 hours.

3. Study Design

This is a single-center, two-site field study with all participants testing at least one product. The control for each test is an untreated person. There is no blinding or randomization employed, since the outcome measures are based on mosquito behavior. Repellent product testing takes place in a field setting using 10 participants (preferably with a 50:50 ratio of males to females, with a minimum of three of either gender) for mosquitoes. Each participant will test a single product at a time (see section 8).

3.1. Study Endpoints

The primary endpoint is the median Complete Protection Time (CPT) of the repellent product. The CPT is defined as the time between application of the repellent product and the occurrence of the first landing in a 5 minute test, followed by a confirmatory landing within 30 minutes.

3.2. Risks and Benefits

Participants will be exposed to biting insects and if bitten may experience some irritation and itching. As this is a field study efforts will be taken to minimize the risk of infection.

- Mosquito sampling will occur in the two sites weekly for a month prior to the tests using two trap types, all mosquitoes captured will be identified and select genera will be submitted for pathogen testing.
- This is a landing study and mosquitoes will be captured before they have chance to bite.
- All participants will be trained in aspirating mosquitoes and spotting mosquito landing behavior.
- All participants will be asked to wear light, loose fitting clothing that fully covers the rest of their body. They will also be provided with a head net.

- All mosquitoes that land will be captured, identified and select genera will be submitted for pathogen testing.
- All *Culex* species captured will be tested for West Nile virus (WNV).
- All *Aedes* species captured will be tested for Zika virus (ZIKV).

All three products contain IR3535, which is moderately irritating to the eyes, but is not irritating to the skin based on dermal studies in rabbits and human volunteers[1]. It may also produce an allergic reaction or irritation of the respiratory tract. Correct handling by the researcher will avoid risks associated with the eyes or respiratory tract. Volunteers will also be excluded if they have a known allergy to any of the product ingredients, or any skin condition which may affect their reaction to the product.

There is insufficient evidence to fully characterize the risk of IR3535 to pregnant or lactating women. Therefore, pregnant women or women intending to become pregnant will not be included in the study.

Other risks associated with participation in this trial include the risks of being outside in a hot humid climate. Precautions will be taken to prevent sunburn, exposing only minimal skin, wearing a hat etc. Water will be provided to prevent dehydration and snacks will be provided to maintain blood sugar levels if necessary.

General risks to participants associated with involvement in this study will be addressed by adhering to ICH GCP[2], the Declaration of Helsinki[3], the Data Protection Act[4] and all applicable regulatory requirements.

There will be no direct benefit to participants. Indirect benefits to society will be improved products for prevention of mosquito biting and pathogen transmission. The results of this study will inform the product labelling.

3.3 Alternatives to Human Study Research

As the objective of this study is to determine the efficacy of a repellent in protecting human beings against bites from mosquitoes it is necessary to complete this testing using human subjects. As human subjects are known to provide a complex combination of thermal, visual and olfactory cues that are attractive to mosquitoes looking to bite and feed, an alternative model is not currently available that will test the repellent in a suitably realistic scenario. The repellent must repel the mosquito in the presence of the attractive host in order to be truly effective. The risks of this research and the steps taken to counteract the risks are described in section 3.2.

4. Participant Entry

4.1. Screening Procedures

Volunteers will be consented prior to any screening procedures being undertaken. Female volunteers of child bearing potential will be asked to take a pregnancy test. Volunteers who do not meet the criteria for eligibility will be excluded.

4.2. Inclusion Criteria

Volunteers will be healthy individuals and chosen based on their insensitivity to the bites in order to limit any itchiness or discomfort.
Volunteers will be included in the study if they meet all of the following criteria:

- Able and willing to give fully informed consent;
- Male or female;
- Aged 18 to 55 years;
- Non-smokers or willing to refrain for 12 hours prior to and during each test;
- Able to understand English

Volunteers will be advised not to apply any cosmetics associated with a strong scent, such as perfume, hand cream, body wash, or scented shampoo. Additionally, volunteers will be asked not to drink alcohol or consume spicy foods, i.e. curries, hot peppers and garlic and to not engage in vigorous exercise for the 12 hours prior to the tests. This will be verified with the participants prior to the commencement of any tests.

Due to the nature of the study, a field study, it is important that all instructions are understood fully and quickly. Therefore, it is essential that the participants understand English. If the study staff are concerned that the participant is not proficient enough in the English language they will not be permitted to continue the study.

4.3. Exclusion Criteria

Volunteers will be excluded from the study if they meet any of the following criteria:

- Suspected or known to be sensitive or allergic to, or phobic of, mosquito bites;
- Participated in an interventional study (other than a biting insect challenge study) in the previous 3 months;
- Participated in a biting insect challenge study in the previous 48 hours;
- Diagnosed with any cardiac or respiratory disorder (whether active or inactive);
- Individuals with localized skin disorders affecting the legs (such as eczema, psoriasis, or atopic dermatitis) or open cuts or scrapes;
- Allergic to any of the test or reference product ingredients;
- Women who are pregnant, nursing or intending to become pregnant;
- Previous anaphylaxis;
- Aware of having a compromised immune system;

- Employees, managers, and spouses of employees of the University of Florida and of the study Sponsor.
- Unable to understand English

4.4. Withdrawal Criteria

Participants can withdraw at any time without giving a reason for withdrawing. Data collected to the point of withdrawal will be used in the analysis of the study, unless the participant requests that their data is not used, in which case it will be removed from the database. Participants may also be removed at the discretion of the Principal Investigator, where continued participation may affect the safety of the participant or where there is a development of any condition which might interfere with study participation.

5. Randomization and Enrolment

Volunteers will be fully informed before the study and it will be made clear that they can withdraw from the study at any time. Volunteers will be given and asked to read the consent form which must be signed before the test begins.

6. Adverse Events

6.1. Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a patient or study participant
Serious Adverse Event (SAE)	A serious event is any untoward medical occurrence that: Results in death Is life-threatening Requires inpatient hospitalization or prolongation of existing hospitalization Results in persistent or significant disability/incapacity Consists of a congenital anomaly or birth defect Other 'important medical events' may also be considered serious if they jeopardize the participant or require an intervention to prevent one of the above consequences.

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the participant or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

6.2. Reporting Procedures

All adverse events and serious adverse events should be reported. Depending on the nature of the event the reporting procedures listed below should be followed. Any questions concerning adverse event reporting should be directed to the Principal Investigator in the first instance.

6.2.1. Non serious AEs

All adverse events should be recorded on the Adverse Event Record Form (Appendix 1) and Adverse Event Monitoring Questionnaire (Appendix 2) and entered into a spreadsheet and stored on a secure drive. All adverse events will be reported to the Sponsor. Depending on the nature of the event the reporting procedures below should be followed, see Appendix 3 for a flowchart of safety reporting.

6.2.2. Serious AEs

Regardless of the relation of the adverse event to study participation, the event must be reported as a serious adverse event if it meets any of the definitions in section 7.1. AE questionnaires meeting the SAE definition will be submitted to the Principal Investigator, Dr Emma Weeks within 24 hours. SAEs that are assessed by the Principal Investigator as being both related and unexpected must be reported to the UF IRB within 5 days of the Principal Investigator becoming aware of the event.

In the case of a severe reaction such as anaphylaxis, a trained First Aider will be called immediately and the volunteer taken to the nearest Emergency Room (ER).

7. Treatments

LivFul Inc. will provide the three products to be tested. The three products have the same active ingredient, IR3535, in a spray, lotion or wipe. Dose determination will be conducted to obtain the typical consumer dose, prior to test commencement (see section 8.4.3.). The product will be applied to the participant's lower leg. The dose will not exceed the maximum daily limit for IR3535 (see Appendix 4 for calculations of the maximum safe dosage of IR3535). Two negative controls will be completed at each time point to monitor mosquito landing rates (see section 8.5.2). The participants will be randomly assigned to either a treatment or untreated control group.

8. Test Methodology

8.1. Field Sites

Field tests for mosquito repellents will be conducted in at least two distinct habitats, most likely a forest or wetland and an urban environment, where the predominant mosquito species differ. The test will most likely be conducted in Alachua county, Florida, USA. This area is outside the current hotspot of ZIKV transmission but in an area of high mosquito abundance and diversity. However, efforts will be made to include a site where *Aedes albopictus* is present.

8.1.1. Site monitoring

Potential sites for testing mosquito repellents will be monitored at least weekly for a month before testing is scheduled. Two trap types will be utilized, a CDC light trap and a BG Sentinel trap. Trapping will occur for 24 hours and processed as described in section 8.1.2. To minimize risks to subjects, field testing will not be conducted where WNV, ZIKV or other mosquito-vectored diseases have been detected within the previous two weeks. The sites will also not be known ZIKV transmission areas.

8.1.2. Mosquito processing

Mosquitoes captured in the pre-test sampling period will be identified by genus and species, and if possible, by subspecies or strain. *Culex* species will be tested for WNV and *Aedes* species will be tested for ZIKV as described in section 8.1.3. Mosquitoes will be pooled by study site and sampling date into groups of 10 by genera and tested following established protocols.

8.1.3. Mosquito pathogen testing

The adult *Aedes* species mosquitoes from field collections will be analyzed to detect ZIKV RNA using reverse transcription and quantitative PCR (RT-qPCR). In a biological safety cabinet, legs from each mosquito body will be removed and placed in clean individual tubes and stored at -80°C for later processing. The RNA will be extracted from each mosquito body using QiAmp viral RNA kit, whose reagents have been shown to inactivate viruses. RNA mixed from pools of 10 bodies will be screened with qRT-PCR reaction using the iTaq™ Universal Probes One-Step kit (BioRad). Primers and a probe specific to ZIKV are designed to the NS2B gene of the ZIKV isolate (accession #KX520666). A mosquito will be considered positive for ZIKV RNA if qRT-PCR reactions show a Cq ≤ 36.[5] Samples that are positive for ZIKV RNA will be further validated using RT-qPCR to amplify the same NS2B gene and sequence analysis performed (Eurofins MWG Operon LLC). If warranted and time and money permits, RNA from individuals in the positive pools can be screened for presence of ZIKV to estimate infection rate of the field collections.

The adult *Culex* species bodies from field collections will be analyzed to detect WNV RNA using already established protocols.[6]

8.2. Test Insects

Mosquito tests will be conducted where more than one species are present. A site will be selected that has an abundance of ZIKV vectors (*Aedes albopictus*), but no previous history of transmission. Landing insects will be aspirated or trapped before and during the test, and labeled with the time of collection. After the field study, collected insects will be identified by genus and species, and if possible, by subspecies or strain. The number in each taxon collected in each time period will be reported.

After identification, mosquitoes will be subjected to analysis to determine the presence or absence of WNV, ZIKV or other disease organisms as described in section 8.1.3. The results of these analyses will be reported to subjects and included in the study report.

8.3. Volunteer enrollment

8.3.1. Recruitment of volunteers

Following approval by the local Ethics Committee and EPA/HSRB, at least 20 informed and consenting volunteers will be enrolled to take part in the study via an approved recruitment email/poster/advert. From this pool of eligible subjects the participants will be selected to be representative of age, gender, race/ethnicity of the general population. Ten informed and consenting participants (at least 3 males and 3 females for each test site and product) will be recruited. In addition, 2 untreated control participants for each test site will be recruited. There will also be six alternates, for a total number of participants of 18. Only participants who are not sensitive to mosquito bites will be involved in this experiment and those taking part are free to withdraw at any time. Up to an additional 6 people could be enrolled as alternates.

8.3.2. Consent and screening

As part of fully informed consent, the participants will review the procedures associated with each exposure interval during the study so they fully understand what will be expected of them on the test day. The following topics will be covered, the content of the informed consent form, provide an outline of the study, discuss subjects' role in the study, discuss the identity and function of the repellent to which they will be exposed, the potential hazards and steps being taken to address them, and the inclusion/exclusion criteria.

Following a detailed review of the participant information sheet and face-to-face meeting, informed volunteers will be asked to provide their written consent to take part in the study. Their eligibility to take part will then be assessed using a participant-completed questionnaire to screen for confounding health conditions that may make them unsuitable for taking part. For females, a negative pregnancy test prior to the training day and every test day is required in order to enrol and maintain enrolment of such a participant. All females will need to confirm that they are not pregnant and do not intend on becoming pregnant throughout the course of the study. See section 8.4.1. for more details.

8.4. Pre-test participant preparation

8.4.1. Pregnancy testing of females

Female subjects must not be pregnant or be breast-feeding. To confirm that participating test subjects are not pregnant, at the beginning of any day when they will be exposed to mosquitoes, female subjects will be required to perform an over–the-counter pregnancy test that will be supplied by the University of Florida. Each female test subject alone, in a bathroom at the testing site, will perform the test. The test subject will initially see the results only. After completion of the pregnancy test, a female employee associated with the study will ask, in a private setting, if the potential subject still wants to participate in the study. If they do, the negative test result will be verified by that employee and relayed to the Study Director (if the Study Director is not the verifying employee). The results will be kept confidential, and will not be disclosed to anyone other than the test subject, the verifying employee, and/or the Study Director. Test subjects will not be required to disclose the results of the test, with the understanding that if they do not, they will not be allowed to participate in the test. The test subject will dispose test results. This procedure will be repeated for each test day in which any female subject participates.

8.4.2. Attractiveness test

Participants will be tested for attractiveness. They will place one arm in a 45 x 45 x 45 cm cage of 78 mosquitoes (*Aedes*) or density equivalent to one mosquito per 1,160 cm^3. If they do not receive five landings in one minute they will be considered not to be sufficiently attractive to mosquitoes and will not be allowed to continue with the study or finish the training. Pathogen-free colony insects will be used that have been in colony for at least 10 years. They will be tested prior to the study to confirm the absence of ZIKV (*Aedes*) and WNV (*Culex*). Pathogen-free colony insects will be used that have been in colony for at least 10 years. The mosquitoes will be tested prior to the study to confirm the absence of ZIKV (*Aedes*) and WNV (*Culex*) as described in section 8.1.3.

8.4.3. Insect landing catch training

Participants will be trained in a screened free-flight cage to identify mosquito landing behaviour and to use aspirators to collect landing insects before they have time to probe or bite. Pathogen-free colony insects will be used that have been in colony for at least 10

years. The mosquitoes will be tested prior to the study to confirm the absence of ZIKV (*Aedes*) and WNV (*Culex*) as described in section 8.1.3.

8.4.3. Dose determination

To estimate a "typical consumer dose" the application of the three products will need to be evaluated prior to the study. Participants allocated to the negative control group will not be involved in dose determination. To estimate a "typical consumer dose" each participant will be asked to apply the repellent lotion or wipe three times to themselves as they would do normally to cover their leg evenly from the top of the sock to the knee. The surface area of the leg also will be calculated. The amount applied in each application by each participant will be calculated by weighing the bottle or wipe before and after application. For the spray, the legs of the participant will be wrapped in gauze "bracelets" of a known area. The bracelets will be weighed before and after application of the spray. The "typical dose" in mg/cm^2 converted to ml/cm^2 skin will be calculated as the mean of three applications by each of the participants and the specific gravity of the test material.

8.5. Test methodology

8.5.1. Subject meeting

Subjects will meet at the Entomology and Nematology Department, University of Florida, Gainesville FL, or other suitable location for application of the repellent. They will be permitted to leave after treatment as long as they agree to return and in the meantime avoid alcohol, tobacco, and scented products (perfume, cologne, hair spray, lotion, soap, etc.). In addition, participants should avoid strenuous exercise and sweating before and during the study, as well as avoiding abrading, rubbing, touching, or wetting the treated area. Transport will be provided to study sites in preparation for the start of the testing.

8.5.2. Subject preparation

Before treatment, a lower leg (ankle to knee), of each participant will be washed with unscented soap and carefully rinsed and dried. With exception of the treated area, the participant head, trunk, and limbs will be covered with light-colored material through which insects cannot bite. Participants should avoid alcohol, tobacco, and scented products (perfume, cologne, hair spray, lotion, soap, etc.) for at least 24 hours before and throughout the test. In addition, participants should avoid strenuous exercise and sweating before and during the study, as well as avoiding abrading, rubbing, touching, or wetting the treated area.

8.5.2. Untreated control participants

Two participants per test day will not be treated with a repellent on any limb. These participants will be fully clothed to prevent mosquito bites. Untreated control participants will monitor the mosquito activity at regular intervals during the test, by counting and collecting

mosquitoes landing on their clothing, to confirm continued acceptable landing pressure. Controls with negative inert substances will not be done to reduce risk to the participants of mosquito biting and pathogen transmission. A positive control will also not be completed, IR3535 is a known efficacious repellent, in this study we are trying to determine the effectiveness (CPT) of the formulations.

8.5.3. Initial landing pressure

As participants will be treated two hours before the start of the test the initial landing pressure will be monitored by the untreated control participants. At least one mosquito landing within one minute should be recorded for the trial to progress.

8.5.4. Product application

The surface area of the lower leg (ankle to knee), will be calculated in order to calculate how much repellent each participant should receive. The “typical consumer dose” of the lotion or spray will be applied to the lower leg. With the wipe, the applied amount will be measured by weighing the wipe before and after use. The repellent will be spread evenly over the lower leg from the ankle to the knee using a single gloved finger to ensure uniform coverage.

8.5.5. Continued landing pressure

Before each exposure period starts, the lower limb of each untreated control participant will be monitored (unexposed) for 1 minute to ensure that at least one mosquito lands for the trial to progress. Insects landing on controls will be collected by mouth aspiration for later identification and labelled with the time of collection.

8.5.6. Subject Placement

Each treated and untreated control participant will be paired with a trained member of staff or another participant. The two untreated controls will be paired together. Each pair will be located at least 3 m/10 ft apart from other pairs.

8.5.7. Exposure period

The treated lower limb of the treated participants will be exposed for 5 minutes. Under supervision of a trained member of staff or another participant, the number and timing of each landing during each exposure period for each participant will be recorded. All landing insects will be collected for identification by aspiration and labelled with the time of collection (before they have chance to probe or bite).

8.5.8. Exposure duration

In order to ensure an acceptable landing pressure throughout the duration of the study, as well as to preserve wellbeing, maintain morale amongst the volunteers, and work within the limits of daylight available, the first two hours of testing will be skipped. Given the typical range of CPT data for IR3535 products[7] it is highly unlikely failure will occur before this time point. Each participant will test all time points. Between time points the repellent will be left on the leg and re-tested every 30 minutes up to 12 hours or until Complete Protection Time (CPT) has been determined. CPT is defined as one landing in the 5-minute test period followed by a second confirmatory landing in the next test period, 30 minutes later, on the treated leg.

8.5.9. Environmental Conditions

The time of day at which subjects are treated and at which exposure to target insects begins and ends will be recorded and reported. Weather conditions (including temperature, relative humidity, cloud cover, precipitation, light intensity, and wind speed) will be monitored periodically throughout the study and reported. Testing will be not be conducted or continued if wind speed exceeds 16 kph/10 mph or if it is raining.

8.6. Follow-up after testing

Participants will be followed up within 72 hours after the test to assess any possible adverse events.

9. Statistics and Data Analysis

9.1. Sample size calculation

9.1.1. Dose determination

Based on preliminary data with a mean of 3.8 and an SD of 3.5, we calculated the sampling error (confidence limit with) for a 95% confidence interval.

Volunteer 1	Replicates 1, 2, 3 Mean	0.52, 0.25, 0.43 0.40
Volunteer 2	Replicates 1, 2, 3 Mean	13.31, 5.33, 3.23 7.29
Volunteer 3	Replicates 1, 2, 3 Mean	3.49, 2.64, 3.49 3.20

The estimation of SD of 3.5 is inflated due to an extreme observation where the participant continued to apply product as the gauze prevented them from feeling as though enough had been applied, so, we expect that a value of 3 is more realistic.

The power calculation below indicates that for a 70% power, with a sample of 11 individuals it is possible to obtain a confidence interval of +/- 1.5 units from the mean under the realistic scenario.

SD	SE	70%	80%
3	1.5	11	13
	2	7	9
3.5	1.5	12	15
	2	8	9

9.1.2 Complete protection time

Based on data from a previously completed field trial of mosquito repellents in Florida[8], where the observed CPT ranged from 2.5 to 5.5 hours with a median of 5 hours, and where 1 out of 8 individuals was censored, a calculation of 95% confidence interval of the median was performed for this study based on a sample size of 10 individuals with the same parameters as above with 1 individual censored (i.e. 10%) at 5.5. hours. This obtained a confidence width of 3 hours. Regardless of the observed data the lower limit of this 95% confidence interval corresponded to the 3rd lowest observed time. Similar calculations were performed for the same sample size, but with 2 individuals censored (i.e. 20%), which resulted in identical results.

9.2. Data analysis

The endpoint for Complete Protection Time will be time to treatment failure for each participant test. Treatment failure is the time at which the product no longer provides complete protection, which is determined as the time at which one landing occurs in a 5 minute period, followed by a confirmatory landing within 30 minutes. The times to treatment failure will be analyzed using Kaplan-Meier Survival functions, and from these the median Complete Protection Time and 95% confidence intervals will be calculated.

Adverse events will be tabulated and included in the study report. Adverse events occurring after the end of participant participation but before the end of the study will be listed separately.

10. Safety and Data Monitoring

10.1. Risk assessment

The Principal Investigator has determined studies of this kind to be “low-risk”. Day-to-day monitoring will be carried out at the study center by a member of the study team with delegated responsibility. A separate monitoring team from *arctec* will monitor the study before participant enrolment, during the study and on study completion.

Safety information regarding the repellent used in the trial have been assessed, material and safety data sheets (MSDS) and labels have been read to be sure they are safe for human use. The active ingredient in the repellent to be tested is IR3535. Participants will be explained the details of the ingredients and what to do if they have a reaction to the product or the mosquitoes after completion of the test.

10.2. Adverse events

Volunteers will be monitored throughout the duration of the tests by investigational staff for any adverse events. If any adverse events related to insect exposure or the repellent product are apparent at any time during the trial, testing will stop immediately and details of how to access treatment will be offered. Participants with known allergies to insect bites or any of the product ingredients will not be eligible to take part. An adverse reaction to an insect bite is defined as a weal greater than 1 cm in diameter that is very red or very itchy.

Within 72 hours after field testing an email will be sent to participants asking them to report any adverse events that might have occurred since the end of testing. Adverse events that occur >72 hours after the end of participation in the trial will be passively monitored.

All adverse events should be recorded on the Adverse Event Record Form (Appendix 1) and Adverse Event Monitoring Questionnaire (Appendix 2) and entered into a spreadsheet and stored on a secure drive. All adverse events will be reported to the Sponsor. Depending on the nature of the event the reporting procedures below should be followed, see Appendix 3 for a flowchart of safety reporting.

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalization but may jeopardize the participant or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious

Regardless of the relation of the adverse event to study participation, the event must be reported as a serious adverse event if it meets any of the definitions in section 7.1. AE questionnaires meeting the SAE definition will be submitted to the Principal Investigator, Dr Emma Weeks within 24 hours. SAEs that are assessed by the Principal Investigator as

being both related and unexpected must be reported to the UF IRB within 5 days of the Principal Investigator becoming aware of the event.

In the case of a severe reaction such as anaphylaxis, a trained First Aider will be called immediately and the volunteer taken to the nearest Emergency Room (ER).

An adverse event which is ongoing at the time of participant withdrawal or completion will be followed up until it resolves or until 30 days after the participant terminates from the study, whichever comes first.

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a patient or study participant
Serious Adverse Event (SAE)	A serious event is any untoward medical occurrence that: Results in death Is life-threatening Requires inpatient hospitalization or prolongation of existing hospitalization Results in persistent or significant disability/incapacity Consists of a congenital anomaly or birth defect Other 'important medical events' may also be considered serious if they jeopardize the participant or require an intervention to prevent one of the above consequences.

10.3. Compensation due to adverse events

If you are injured as a direct result of your participation in this study, the Sponsor will pay for all reasonable and necessary medical expenses required to treat your injury, as long as:

1. The injury occurs during your participation in the study.
2. The injury results directly from the study product or study-required procedures.

The Sponsor and the Principal Investigator will determine whether your injury is related to your participation in this study.

No additional compensation is routinely offered. The Principal Investigator and others involved in this study may be University of Florida employees. As employees of the University, they are protected under state law, which limits financial recovery for negligence.

Please contact the Principal Investigator Dr. Emma Weeks on 352-870-4327 if you experience an injury or have questions about any discomforts that you experience while participating in this study.

10.3. Data monitoring

With the exception of the Volunteer Questionnaire, which is completed by the participant, all data collected will be recorded in the case report form (CRF) and signed by the person completing the CRF. The CRF is considered to be source data.

Data to be collected are: Participant number and date of visit on every page, confirmation of informed consent, date of birth, eligibility details, mosquito attraction details, test visits (eligibility checklist, product details, product application details, field testing data (time, fitness check, No. insects landing) and adverse event monitoring.

Raw data from the CRF are then entered into an Excel spreadsheet for analysis and saved on a secure drive. Data will be double entered and verified to ensure accuracy. CRFs will be kept in locked storage.

Information in the database for each test will be linked to a relevant SOP, risk assessment, contract, and files of statistical analysis.

11. Regulatory Issues

11.1. Ethics approval

The PI at the Entomology and Nematology Department (University of Florida, Gainesville, FL, USA) will obtain ethical approval from the UF Institutional Review Board (IRB).

11.2. Consent

Consent to enter the study must be sought from each volunteer only after a full explanation has been given, and time allowed for consideration. Signed volunteer consent will be obtained. The right of the participant to refuse to participate without giving reasons must be respected.

11.3. Confidentiality

Participants' identification data will be required for the enrolment process. The Trial Coordination Centre will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

11.3.1 How will health information be collected, used and shared?

The Principal Investigator will create, collect, and use protected health information or PHI. This information can be gathered from the participants and any tests explained in previous sections of this protocol. This information will be obtained during study visits and telephone calls.

More specifically, the following information may be collected, used, and shared with others:

- Name will be collected and assigned a coded subject identifier based on gender, such as Subject M1, F2, F3, M4, etc.
- Age will be requested because participation of those under the age of 18 or over the age of 55 is prohibited for this study.
- Gender may be reported with the data as the coded identifier above, where M is for male and F is for female.
- Information about health including: general health, allergies, pregnancy (if applicable), insect bite history, and participation in other studies.
- phone number and email address
- social security numbers: for compensation payments

This information will be stored in locked filing cabinets or on computer servers with secure passwords, or encrypted electronic storage devices.

If limited data sets are created and used, agreements between the parties creating and receiving the limited data set will be obtained.

11.3.2. For what study-related purposes will PHI be collected, used, and shared with others?

PHI may be collected, used, and shared with others to make sure participants are eligible to take part in the research, through participation in the research, and to evaluate the results of the research study.

More specifically, PHI may be collected, used, and shared with others for the following study-related purpose(s):

- The purpose of this study is to evaluate the repellency of novel and commercial repellents against mosquitoes that bite humans and other animals. Some of the results from these studies will be averaged and reported and published in scientific journals.
- Some results from these studies may be kept confidential so that only the PI, their staff and the repellent manufacturer are aware of the results.
- Once this information is collected, it becomes part of the research record for this study.

11.3.3. Who will be allowed to collect, use, and share PHI?

Only certain people have the legal right to collect, use and share the research records, and they will protect the privacy and security of these records to the extent the law allows. These people include:

- the study Principal Investigator (listed in question 3 of this form) and research staff associated with this project.
- other professionals at the University of Florida or Shands Hospital that provide study-related treatment or procedures.
- the University of Florida Institutional Review Board (IRB; an IRB is a group of people who are responsible for looking after the rights and welfare of people taking part in research).

11.3.4. Once collected or used, with whom may PHI be shared with?

PHI may be shared with:

- the study Sponsor (listed in Part 4 of this form).
- United States agencies who are responsible for overseeing research, such as the Food and Drug Administration, the Department of Health and Human Services, the Environmental Protection Agency, and the Office of Human Research Protections.
- Government agencies who are responsible for overseeing public health concerns such as the Centers for Disease Control and federal, state and local health departments.

Otherwise, research records will not be released without permission unless required by law or a court order. It is possible that once this information is shared with authorized persons, it could be shared by the persons or agencies who receive it and it would no longer be protected by the federal medical privacy law.

11.3.5. How long will PHI be used and shared with others?

PHI will be used and shared with others until the end of the study. If the consent form is signed then the participant is authorizing researchers to collect, use and share PHI. Participation is not permitted in this research unless you allow the collection, use and sharing of your protected health information by signing this consent and authorization.

Participants can revoke authorization at any time before, during, or after your participation in this study, by giving a written request with a signature on it to the Principal Investigator. If authorization is revoked, no new information will be collected. However, information that was already collected may still be used and shared with others if the researchers have relied on it to complete the research.

11.4. Sponsor

LivFul Inc. is the Sponsor. Address: 2972 Webb Bridge Rd, Alpharetta, GA 30009, USA

11.5. Funding

LivFul Inc. will fund the study. Participants will be offered up to $120 per test ($10 per hour; 6 visits in total) and up to $60 for attending the consenting, enrollment and training visits, to defray the cost of attending. If they test one product at one site, this is a total of $180. If they test all three products at both sites (6 complete tests) this is a total of $780.

Visit	Reason			Compensation
1	Consenting			$20
2	Enrollment and Dose Determination			$20
3	Mosquito attractiveness and training			$20
4	Site 1	Product 1	Test 1 (1-4 days)	$120
5	Site 1	Product 2	Test 2 (1-4 days)	$120
6	Site 1	Product 3	Test 3 (1-4 days)	$120
7	Site 2	Product 1	Test 4 (1-4days)	$120
8	Site 2	Product 2	Test 5 (1-4 days)	$120
9	Site 2	Product 3	Test 6 (1-4 days)	$120
			Total	$780

11.6. Record retention

Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period.

12. References

1. Safety Profile Insect Repellent IR3535®. EMD Performance Materials, 2016.

2. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (1996) 'ICH Harmonised Tripartite Guideline E6(R1): Guideline for Good Clinical Practice'.

3. World Medical Association Declaration of Helsinki (1964) 'Ethical Principles for Medical Research Involving Human Subjects'.

4. Data Protection Act 1998.

5. Smartt, CT, TMS Stenn, T Chen, MG Teixeira, EP Queiroz, LSD Santos, GAN Queiroz, KR Souza, LK Silva, D Shin & WJ Tabachnick. 2017. Evidence of Zika virus RNA fragments

in *Aedes albopictus* (Diptera: Culicidae) field collected eggs from Camaçari, Bahia, Brazil. J Med Entomol (ACCEPTED).

6. Smartt, C.T., Shin, D., Anderson, S.L. 2016. The Effect of West Nile Virus Infection on the Midgut Gene Expression of Culex pipiens quinquefasciatus Say (Diptera: Culicidae). Insects 7, 76; doi:10.3390/insects7040076

7. Merck. The Effectiveness of IR3535® Against Mosquitoes. http://us.ir3535.com/en/effectiveness_of_ir3535/against_mosquitoes/against_mosquitoes.html

8. EPA (2016). Science Assessment: Field Testing of S.C. Johnson Personal Mosquito Repellent Mark-4 Product to Support the Use of the EPA Repellency Awareness Graphic https://www.epa.gov/sites/production/files/2016-11/documents/final_mark_4_study_science_and_ethics_presentation.pdf

Appendix 1. Adverse Event Record Form

Study Title:

Volunteer reference number	Date	Side effect/adverse event	Related to the product	Comments	Action taken
			Y/N/ don't know		

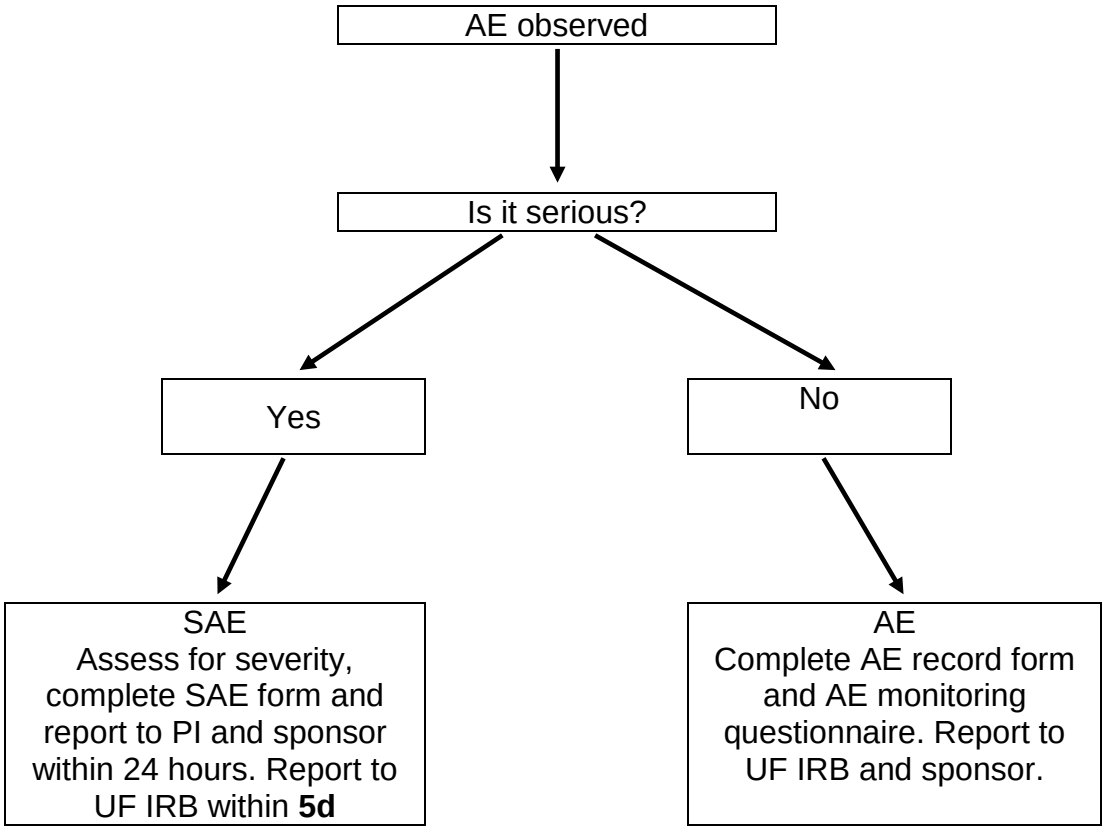
Appendix 2: Adverse Event Monitoring Questionnaire

To be completed by person who experienced the Adverse Event	
Name (first name SURNAME)	
Date of birth dd/mm/yyyy	
Phone number	
Mobile number	
E-mail address	
What kind of adverse event did you experience? e.g. skin rash, burning sensation, severe allergic reaction	
How long did the adverse event last?	
How serious was the event? mild / moderate / severe / life threatening	
Did you take any action to resolve the event?	
Was any treatment required?	
Did you visit A&E? Please enter details	
Did you stay in hospital overnight? Pease enter details (no. nights/admission)	
Outcome unresolved / resolved / resolved with sequel	
To be completed by trial manager	
Participant ID	
Study title	
Study code	
Type of study e.g. repellent, impregnated clothing, after-bite cream	
Exposure type e.g. chemical, mosquito bites, bed bug bites	
Exposure area e.g. forearm, legs, hands	
Active ingredient	
Report date dd/mm/yyyy	
Other event Please enter details	

Likelihood of Adverse Event being related to study unrelated / unlikely / possible / probably / definite	
Serious Adverse Events	
Was the event serious? yes / no	
Admitted to Intensive Care Unit? yes / no	
Seriousness criteria (please tick)	
life threatening	
required hospitalisation	
prolonged hospitalisation	
congenital anomaly	
disabling/incapacitating	
important medical event	
required intervention to prevent impairment or damage	
Fatal	
If fatal, date of death dd/mm/yyyy	
Primary cause of death	
Was a post-mortem performed? yes / no	
Date adverse event become serious dd/mm/yyyy	
Possible contributing factors to SAE other than study participation or underlying disease being studied Please give details	
None apparent	
Concurrent illness, disease or other external factors	
Concurrent medication	
Study procedure	
Accident, trauma, or other external factors	
Other	
Relevant concomitant medication at time of SAE yes / no – if yes please provide details	
Treatments/procedures for SAE yes / no – if yes please provide details	

Relevant medical history (include only relevant past or concurrent medical disorder, surgeries, etc that might help explain the SAE) yes / no – if yes please provide details	
Relevant laboratory testing yes / no – if yes please provide details	
If relationship to study participation was unrelated, provide causality Please give specific details	
Discontinuation of study participation	
Concurrent disorder	
Concomitant medications	
Other	
If action taken with study participation, was study interrupted or discontinued? Provide date (dd/mm/yyyy)	
Did SAE abate after study was stopped? yes / no / not applicable / unknown	
Did SAE reoccur after reintroduction of study participation? yes / no / not applicable / unknown	
Narrative/Comments Please describe the SAE including a chronological clinical presentation and evolution of the SAE and associated signs/symptoms	
Please submit this questionnaire to Dr Emma Weeks. SAEs that are assessed by the PI as being both related and unexpected must be reported to the UF IRB within 5 days of the PI becoming aware of the event.	

Appendix 3: Flowchart for Safety Reporting



Appendix 4: Calculations of Maximum Safe Dosage of IR3535

Participant	AEL* (mg/kg bw/day)	Weight** (kg)	Max Internal Dose IR3535® (mg/day)	Dermal Absorption* (%)	Max External Dose IR3535® (mg/day)
Adult Male	5	73.80	369	14	2635.71
Adult Female	5	60	300	14	2142.86

* Accepted exposure level (AEL) and Dermal Absorption taken from: Regulation (EU) nº528/2012 concerning the making available on the market and use of biocidal products: Evaluation of active substances Assessment Report: Ethyl butlyacetylaminopropionate Product-type 19 (insect repellent) (2013) (CA-Dec13-Coc.3.4a – IR3535 draftAR.docx).

** Weight taken from: U.S. Environmental Protection Agency (EPA). (2011) Exposure Factors Handbook: 2011 Edition. National Center for Environmental Assessment, Washington, DC; EPA/600/R-09/052F.

Participant	Max External Dose IR3535® (mg/d)	Product: % IR3535®	Max External Dose Product (mg/d)	Lower Leg Surface Area (cm^2)	Product Application Rate (mg/cm^2)	Amount of Product Applied to Lower Leg (mg/cm^2)	Amount of Product Applied as % of Max External Dose (IR3535® or Product)
Adult Male	2635.71	15%	17571.43	1044	1.67	1743.48	9.92
Adult Male	2635.71	17%	15504.20	1044	1.67	1743.48	11.25
Adult Male	2635.71	20%	13178.57	1044	1.67	1743.48	13.23